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CHEMICAL, CYTOLOGICAL AND GENETIC EVIDENCE FOR THE HYBRID ORIGIN OF ASTER BLAKEI (PORTER) HOUSE[1]

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The genus *Aster* is large and polymorphic, and many authors have cited the taxonomic complexity of this group (Anderson, 1929; Shinners, 1941; Rosendahl & Cronquist, 1949). Fernald (1950) suggested that some of the variation causing this complexity was due to hybridization, and this was anticipated by Wetmore & Delisle (1939), and verified by Avers (1953a), and Uttal (1962). The work of Avers on asters of the Heterophylli series demonstrated that many species could be crossed in cultivation, producing hybrids (Avers, 1953a). However, she noted the absence of such crossing in natural populations. She observed that the barriers to hybridization in nature were primarily ecological, relating to the absence of hybrid habitats (Avers, 1953b).

A recent study by Pike (1970) presented morphological and geographical evidence indicating that *Aster Blakei* (Porter) House was of hybrid origin from *A. acuminatus* Michx, and *A. nemoralis* Ait. The morphological data fur-

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ther indicated that introgression might be occurring in the direction of *A. nemoralis*.

This paper considers further the origin of *A. Blakei*, utilizing cytological, genetic and chemical techniques. The chemical studies were conducted using thin-layer chromatography of phenolic compounds. These compounds were used as markers in detecting natural hybridization. The genetic techniques involved crosses and backcrosses of the putative parents, *A. acuminatus* and *A. nemoralis*. Self-pollinations also were made to synthesize an F_2 population. The cytological approach involved studies of mitotic chromosomes of the parental taxa and the F_1 hybrid, including specimens of *A. Blakei* cultivated in the greenhouse. Pollen stainability of the parents, F_1 hybrids, backcrosses and F_2 hybrids also were observed.

The application of analytical chemistry to taxonomic studies has been increasingly successful as techniques have become more refined. The work of Turner & Alston (1959) and Alston & Turner (1962) on hybridization in *Baptisia* started the recent emphasis in chemotaxonomy. The species specific compounds of two putative parents in this genus both appeared in suspected hybrids. This has become a principle of phenolic biochemical systematics: the phenolic compounds found in hybrids represent a summation of the species-specific phenolic compounds found in the parents (Alston, 1965 and Harborne, 1968). Utilizing this principle, other workers have confirmed hybridization in various genera. For examples, see Smith & Levin (1963) in *Asplenium*, Jaworska & Nybom (1967) in *Saxifraga*, Olden & Nybom (1968) in *Prunus*, Fahselt & Owenby (1968) in *Dicentra*, and Walker (1969) in *Petalostemon*. The technique has been useful in demonstrating introgression in *Iris* (Carter & Brehm, 1969) and in confirming an instance of intergeneric hybridization (Crang & Dean, 1971).

Very few chemosystematic studies in *Aster* have been published to date. A preliminary survey of phenolics by Turner & Mabry (1964) on various members of the Asteraceae cited only one species of *Aster*. The research of Abra-

hamson & Solbrig (1970) revealed no species-specific phenolics in the Heterophylli series and the authors advised against the use of phenolics in taxonomic considerations of this group. There have been no attempts to consider hybridization in Aster from a chemical point of view.

SELECTION AND MORPHOLOGICAL ANALYSIS OF COLONIES

Living material of *A. acuminatus* and *A. nemoralis* was provided by Dr. Pike from natural habitats in eastern Maine and brought to the greenhouse at the University of New Hampshire in the autumn of 1969. Specimens of *A. Blakei* were collected by the senior author near the southern shore of Lake Ossipee in New Hampshire during the same season. The specimens were potted in a mixture of peat and vermiculite and placed in a house maintained at 24° C. When cold treatment was necessary, the plants were moved to a room maintained at 10°C. This treatment was applied from late November to mid-January of each year.

Specimens subjected to morphological and chemical analysis were collected from three main areas. The first collection of twenty-five specimens came from the southern shore of Lake Ossipee in New Hampshire. A second collection of sixteen plants came from the southern shore of Lake Winnisquam in New Hampshire. A final collection of fifty-four plants came from Great Wass Island in Washington County, Maine. This latter collection consisted of a total of fourteen specimens of *A. nemoralis* and *A. acuminatus* in discrete colonies and forty specimens of *A. nemoralis*, *A. Blakei* and *A. acuminatus* collected at Ponds Point on the eastern tip of the island facing the Gulf of Maine. *Aster acuminatus* was collected from colonies in a wooded area of high elevation. *Aster nemoralis* was collected from colonies in a small bog in the center of the island. These asters are clonal. Their stoloniferous habit is a characteristic of the species (Fernald, 1950). The clones at Lake Ossipee and Lake Winnisquam were well defined. One ramet was sampled from each clone at these locations. The clones were

not well defined at Great Wass Island. Sampling at this location was based on morphological diversity throughout the area of the population. The plants collected were all scored utilizing the hybrid index described in detail by Pike (1970).

The morphological analyses of specimens collected from these sites are summarized in Figure 1. Plants scoring 0-4 were designated as *A. nemoralis*, 8-19 as *A. Blakei* and 25-30 as *A. acuminatus* (Pike, 1970). The population at Lake Winnisquam was essentially a variable population of *A. Blakei* (Figure 1a). The population at Lake Ossipee indexed as *A. nemoralis* or *A. Blakei* (Figure 1b). The populations at Great Wass Island contained the parental taxa in discrete colonies (Figure 1c), and all three taxa together in a local population at Ponds Point (Figure 1d). Figure 1e summarizes the data in Figures 1c and 1d.

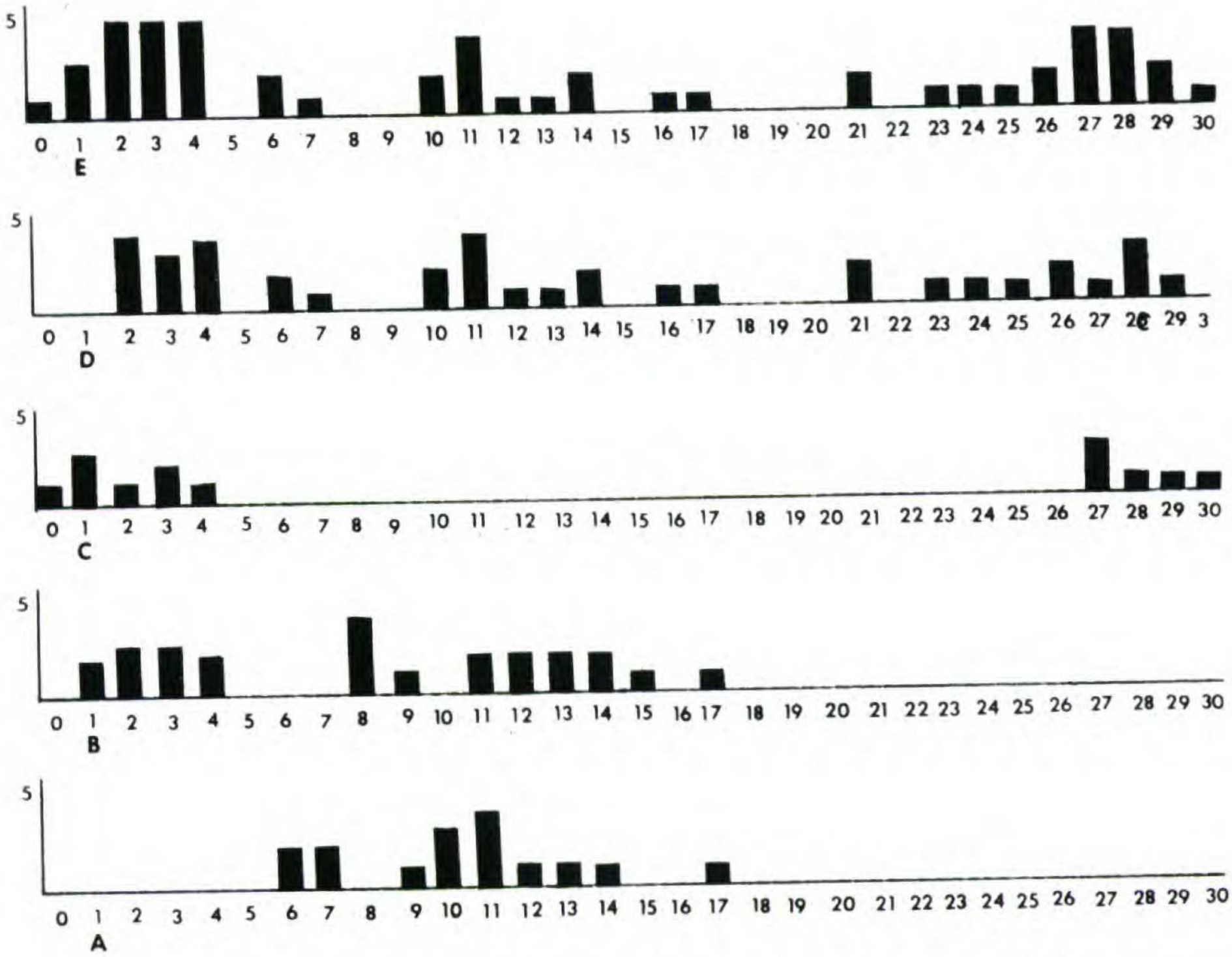


Fig. 1. Morphological hybrid index of Asters at (a) Lake Winnisquam (b) Lake Ossipee and (c, d, e) Great Wass Island.

CHEMICAL DETECTION OF HYBRIDIZATION AND INTROGRESSION

For chemical analyses, fresh basal leaves were shredded at the collection site into 10 ml. of methanol containing 1% 1N HCl. The extracts were stored in the dark for 48 hours at room temperature and then concentrated to approximately 1 ml. by evaporation with the aid of a hand dryer. Paper chromatography gave poor resolution in a comparative test with thin-layer chromatography. In contrast, thin-layer chromatography required only a small amount of extract and gave excellent resolution. The aluminum-backed thin-layer plates were prepared by EM Reagents Division, Brinkman Instrument, Westbury, New York.

Ten microliters of the sample were spotted on one corner of a cellulose plate and chromatographed in the first dimension for 6-8 hours with n-butanol, glacial acetic acid and water (6:1:2). After drying overnight, the plate was chromatographed for 2-2 1/2 hours in the second dimension with 10% acetic acid containing 0.1% sodium acetate. After drying, the plate was viewed under visible and ultraviolet light. Colors of each spot under both conditions were recorded. These colors were again recorded after exposure to ammonia vapor. The plates were subsequently treated with spray reagents recommended by Block, *et al.*, (1958) to distinguish among spots and to determine if these were phenolics. The sprays were: 1% alcoholic ferric chloride; 1% aqueous basic lead acetate; 1% aqueous lead acetate; 1% aqueous sodium carbonate; 1% alcoholic aluminum chloride and Benedicts reagent. Also, diazotized sulfanic acid was applied according to the specifications of Smith (1960). It was discovered that 1% aqueous lead acetate gave good distinctive colors under longwave ultraviolet light. Each spot was labeled with a number for identification on the basis of color of fluorescence, color reactions and position on the plate. The relative location of each spot to each other also was used as a criterion of identification. No attempts were made to determine the chemical structure of these compounds.

A premliminary survey of the phenolics present in the leaves of *A. nemoralis*, *A. acuminatus* and *A. Blakei* collected by Pike and the senior author revealed differences between the number of phenolics at various developmental stages (Hill, 1972). This source of variation was eliminated by studying the phenolics only at the time of full flowering.

Chromatographs of the specimens collected from Lake Ossipee, Lake Winnisquam and Great Wass Island yielded numerous spots identified as phenolic compounds by the following criteria: (a) behavior in the developing solvents (Seikel, 1962); (b) reaction to sulfanilic acid; (c) colors under UV light before and after exposure to ammonia (Al-

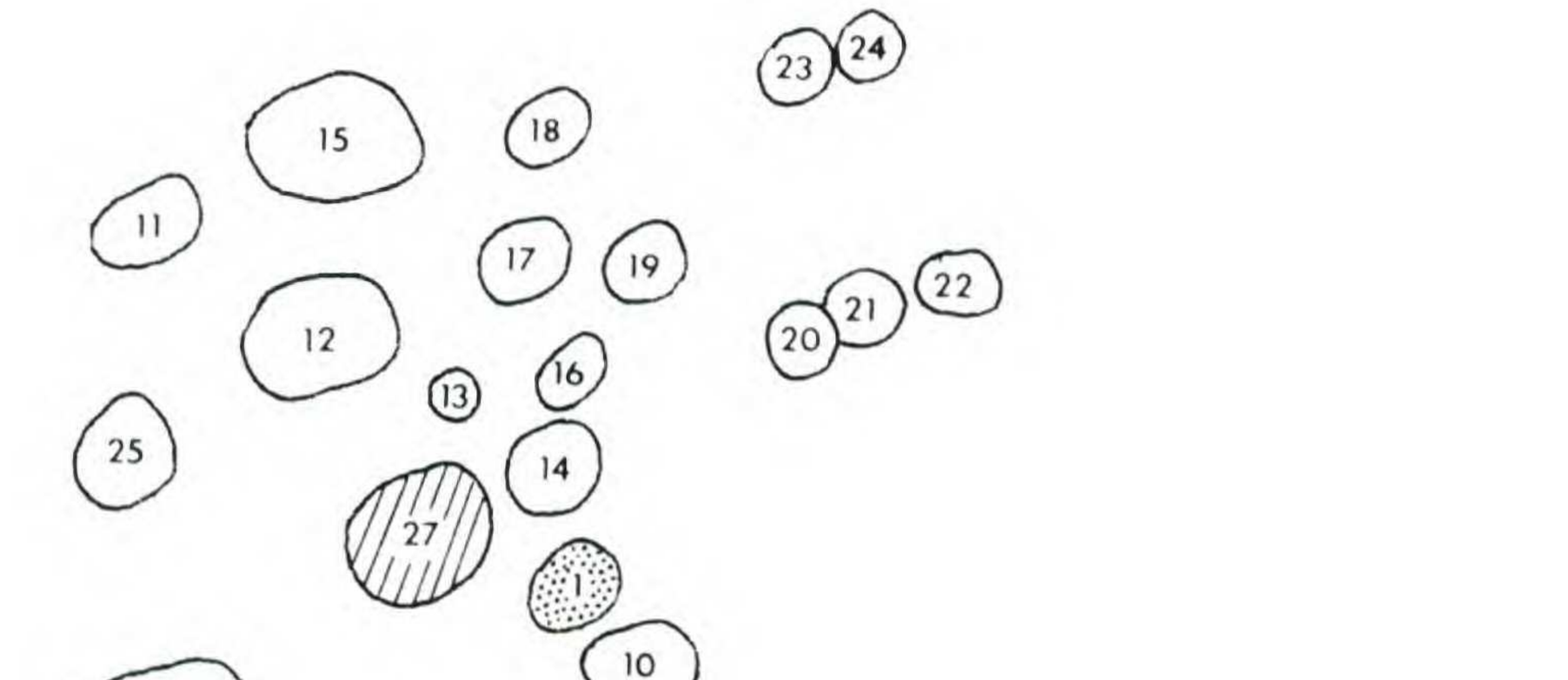


Figure 2. Phenolic profile of *Aster Blakei*, *A. nemoralis*, and *A. acuminatus*. Spots 1, 2, 3, and 4 are specific to *A. nemoralis;* spots 26, 27, and 28 are specific to *A. acuminatus*. The remaining spots are common to all three taxa.

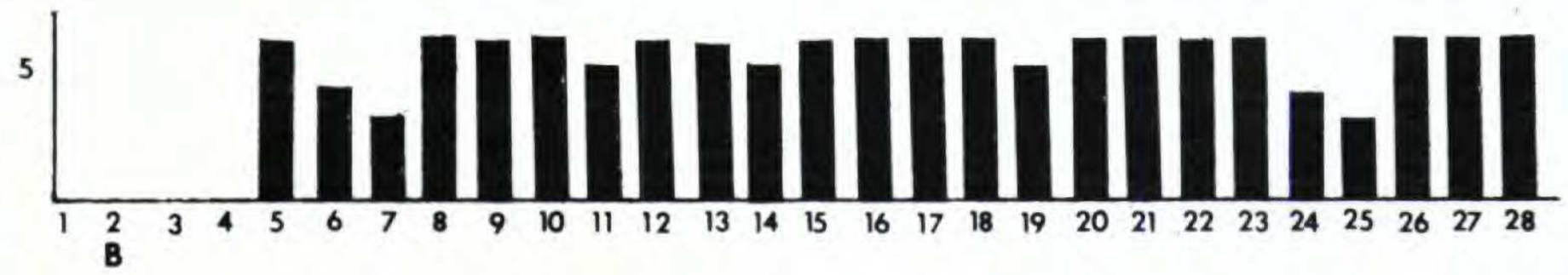


Figure 3. Frequency of occurrence of phenolics characteristic of (a) *Aster nemoralis* and (b) *A. acuminatus* in discrete colonies on Great Wass Island. Numbers refer to spots identified in Figure 2.

ston, 1967); and (d) color reaction to phenolic-specific sprays (Block, *et al.*, 1958). Twenty-eight spots were chosen as diagnostic of these asters because of their high frequency of occurrence or because of their diagnostic value in identifying the parental taxa.

The results of the chemical analysis of the Great Wass Island populations indicate that the parental taxa could be identified on a chemical basis alone. *A. nemoralis,* represented in Figure 1c, contained four compounds which were species-specific. These were compounds numbered 1, 2, 3, and 4 shown in Figure 2. *A. acuminatus,* represented in Figure 1c, contained three compounds which were species-specific. These were compounds numbered 26, 27, and 28 in the phenolic profile in Figure 2. A histogram (Figure 3) of the frequency of occurrence of phenolics in the parental taxa in discrete colonies shows that twenty-one compounds were common to both species. The remaining seven phenolics clearly separate the parental species.

A chemical analysis of the Ponds Point population on Great Wass Island revealed that the parental taxa maintained a similar chemical integrity (Figures 4a, c). *Aster*

Blakei contained a summation of the compounds specific to both parents but did not contain any new species-specific compounds (Figure 4b). The morphological and chemical evidence thus suggests that *A. Blakei* at Ponds Point originated as a hybrid of *A. acuminatus* and *A. nemoralis*. It is clearly a chemical and morphological intermediate of the parental taxa. It is particularly significant that the three taxa could be identified on a chemical basis alone.

The chemical analyses of *A. Blakei* at Lake Winnisquam and Lake Ossipee revealed similar phenolic profiles when compared to *A. Blakei* at Ponds Point. There were differences in the frequency of occurrence of some compounds.

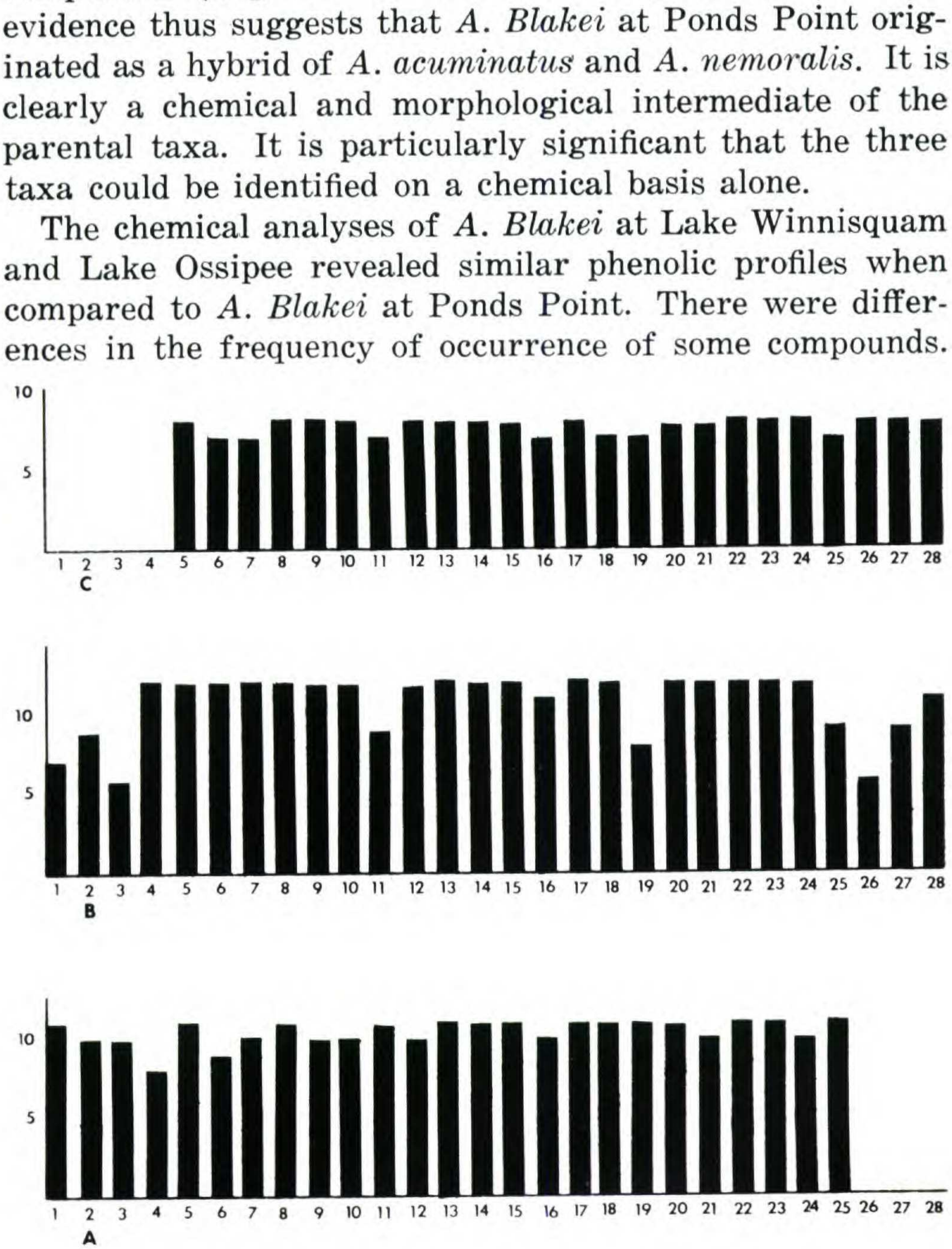


Figure 4. Frequency of occurrence of phenolics characteristic of (a) *Aster nemoralis* (b) *A. Blakei* and (c) *A. acuminatus* at Ponds Point, Great Wass Island. Numbers refer to spots identified in Figure 2.

Table 1. Frequency (in %) of occurrence of compounds diagnostic of *Aster nemoralis* and *A. acuminatus* in relation to morphology in the population at Lake Ossipee, New Hampshire.

Morphological Index	Compound Number				
	2	3	26	27	28
1	50		100		100
2	33	33	100		100
3	67		67		100
4			50		100
8			75	25	75
9			100	100	100
11			100	50	100
12			100	100	100
13			100	100	100
14			100	100	100
15			100	100	100
17			100		

The F_1 hybrids synthesized in the greenhouse demonstrated a high frequency of occurrence of *A. accuminatus* compounds in F_1 hybrids resulting from *A. nemoralis* female parents. The data on the asters collected from the Lakes and from the greenhouse are presented elsewhere (Hill, 1972).

There was evidence for introgression at Lake Ossipee. Table 1 was constructed to show the relationship between chemical and morphological data using a method devised by Levin (1967). High frequencies of *A. acuminatus* compounds numbered 26 and 28 were found in plants which indexed as *A. nemoralis*. Specimens identified as *A. Blakei* contained only *A. acuminatus* phenolics. This suggests that introgression into *A. nemoralis* has been occurring at Lake Ossipee. A pictorialized scatter diagram was constructed according to the methods of Anderson (1949). The diagram was determined from the data on serrate vs. entire

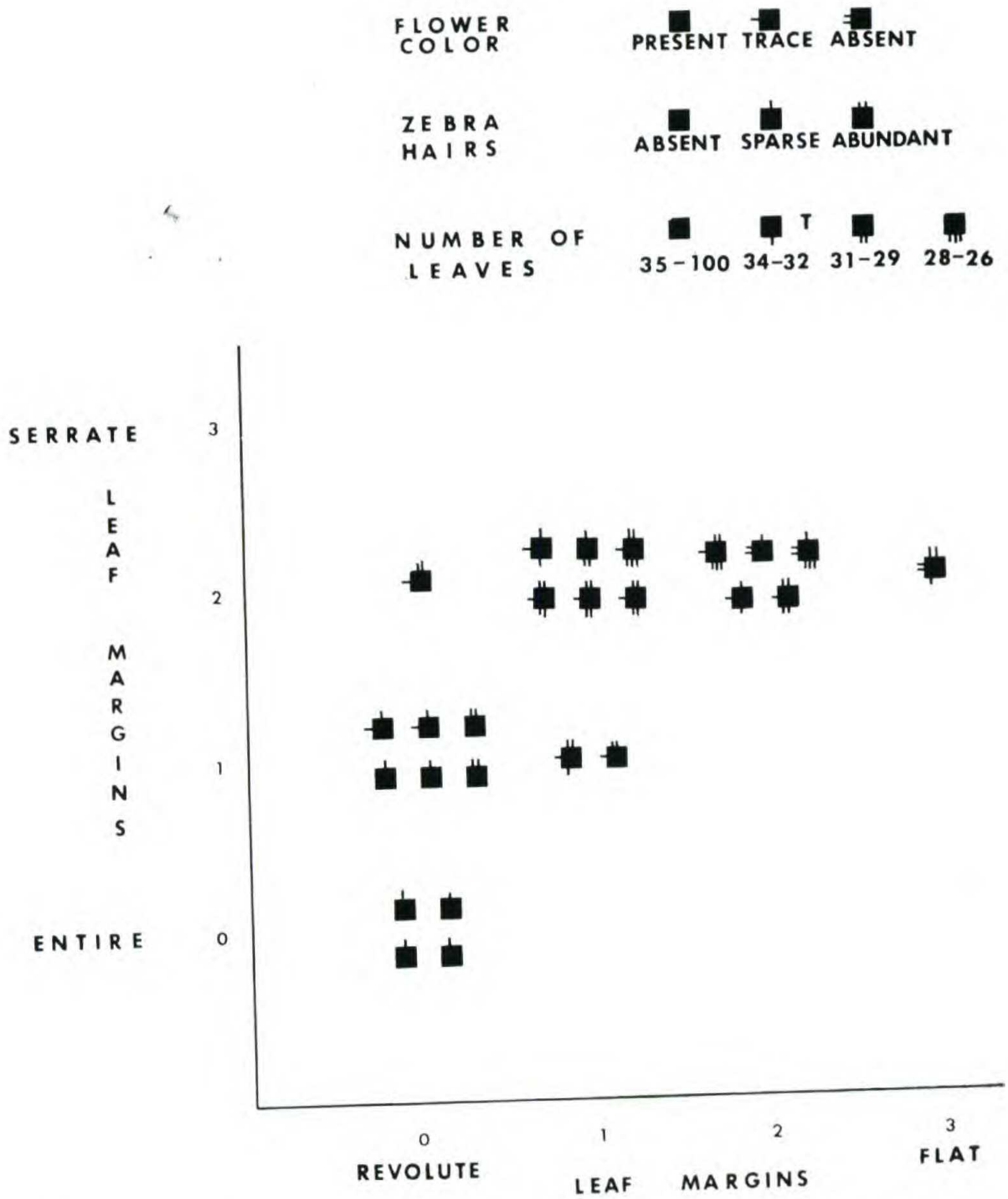


Figure 5. Pictorialized scatter diagram of asters at Lake Ossipee.

leaf margins, revolute vs. flat leaf margins, zebra hairs, flower color and leaf number (Figure 5). Zebra hairs and serrate leaf margins, which are characteristics of *A. acuminatus*, were present in many plants indexed as *A. nemoralis*. This observation provides morphological evidence for the introgression of *A. acuminatus* into *A. nemoralis* at Lake Ossipee. *Aster acuminatus* was not present in this population. The habitat was a disturbed one which was located in an area of land development. There were woody habitats which could have supported *A. acuminatus*, but new roads cut off drainage and many of these areas were too moist and swampy to sustain *A. acuminatus*. It is suggested that the environmental disturbance removed this taxon, leaving *A. Blakei* to cross with *A. nemoralis*. The result is the variable population found there today.

GENETICS

Specimens of *A. acuminatus* and *A. nemoralis* which were collected by Pike were crossed in the fall of 1970 and spring of 1971. The seeds from the fall 1970 crosses were germinated the following spring. These F_1 hybrids were then backcrossed to their parents. Crosses also were made between various F_1 hybrids at that time. The resulting backcross and recombinant progenies were grown during the spring of 1972. Crosses were performed in an insect-free house by rubbing the heads of two plants together when most of the flowers in a head were open and shedding pol-

Table 2. Seed set of the cross *Aster nemoralis* × *A. acuminatus* during two flowering seasons.

Season	Female Parent	Total Flowers Examined	Good Seed	Seed Set (%)
Fall, 1970	*A. nemoralis*	1180	205	17.4
	A. acuminatus	1191	30	2.1
Spring, 1971	*A. nemoralis*	2426	302	12.5
	A. acuminatus	2297	26	1.1

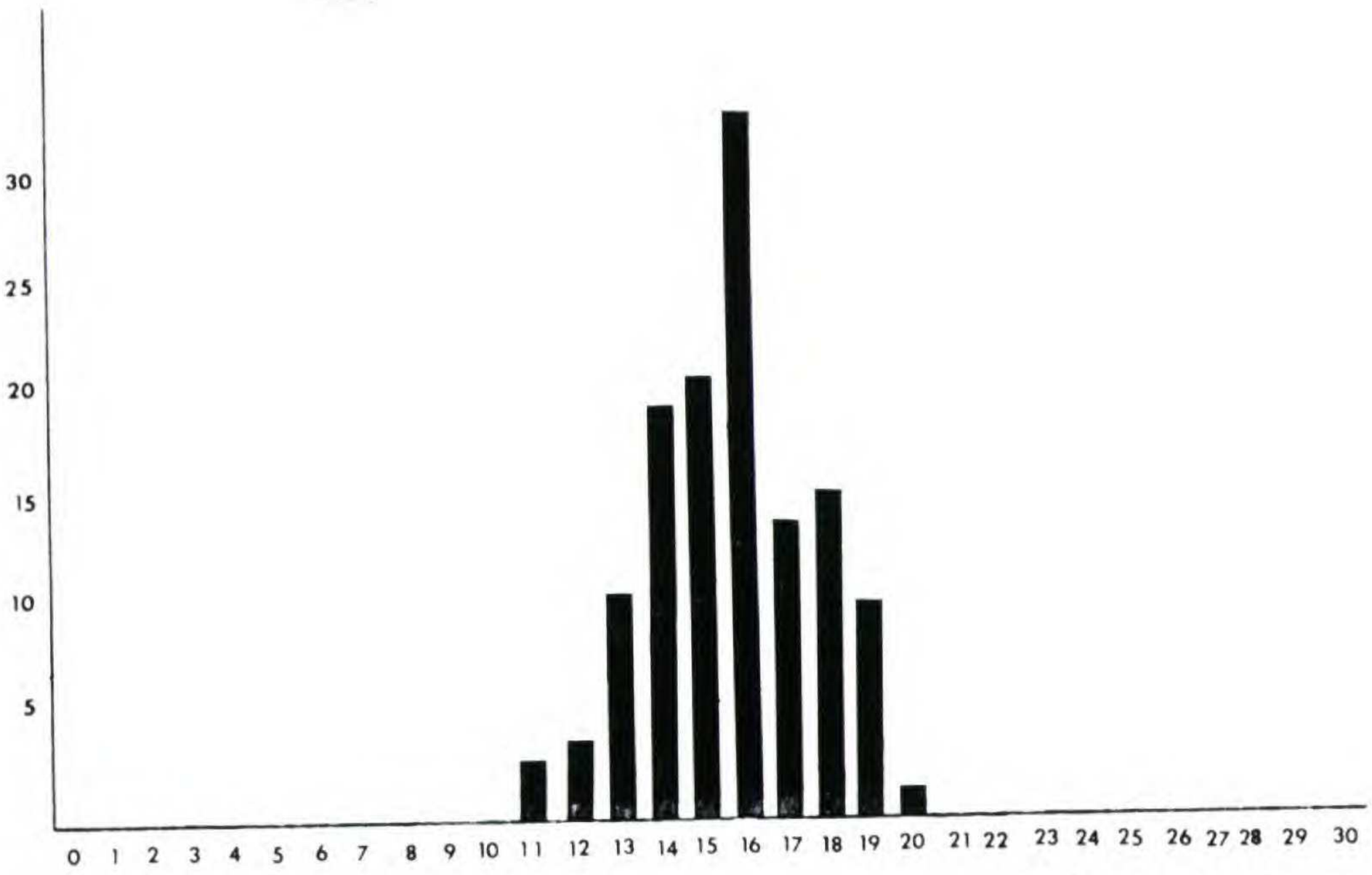


Figure 6. Morphological hybrid index of the F_1 hybrids of the cross *A. nemoralis* × *A. acuminatus*.

len. Seeds from the crosses were stored at room temperature. Before germination they were subjected to a six to seven week treatment at 8°C. in Petri dishes containing moist filter paper. After this cold period, seeds were germinated at 24°C. in a peat-vermiculite mixture covered with ground sphagnum in pots enclosed in plastic bags. Ten days after germination the bags were removed.

Crosses made between *A. nemoralis* and *A. acuminatus* in the fall of 1970 resulted in seed set that was high when *A. nemoralis* was the female parent (Table 2). The same response was obtained in the spring of 1970 using parents from a wider geographic source. The reason for this result probably rests in the presence of a maternal barrier to pollination or fertilization in *A. acuminatus*.

The hybrid index of Pike (1970) was used to score 168 F_1 hybrids. The results are represented in Figure 6. These specimens were morphologically intermediate and fell within the range assigned to *Aster Blakei* by Pike. Representative specimens of *A. Blakei* collected from Lake Ossipee and

Figure 7. *Aster Blakei* from Lake Ossipee (right) and an F_1 hybrid of the cross *A. nemoralis* × *A. acuminatus* (248, left).

Figure 8. *Aster Blakei* from Lake Ossipee (right) and an F_1 hybrid of the cross. *A. nemoralis* × *A. acuminatus* (206, left).

F_1 hybrids from the cross *A. nemoralis* × *A. acuminatus* are compared in Figures 7 and 8. The morphological similarities are quite obvious. Many of the F_1 hybrids also resembled the specimens of *A. Blakei* collected from Lake Winnisquam, Lake Ossipee and Great Wass Island. The F_1 hybrids were morphologically uniform for most characters. Coefficients of variation calculated for the ten characters employed in the hybrid index of Pike (1970) indicated the most variable characters to be internode length, the number of bracts subtending the peduncle and the number of heads (Hill, 1972).

Backcrosses of the parents with the F_1 hybrid resulted in good seed set and seed germination (Table 3). The maternal barriers in *A. acuminatus* that existed for *A. nemoralis* pollen did not exist for pollen from the F_1. The crosses made within the hybrid population also resulted in good seed set and seed germination, although germination was

Table 3. Seed set and seed germination from crosses between the parental taxa and their F_1 hybrid, Spring, 1971.

Female Parent	Flowers Examined	Good Seed	# Germ.	Seed Set (%)	% Germ.
A. acuminatus	1267	345	158	27	46
F_1 Hybrid	1213	476	310	39	65
A. nemoralis	1594	428	217	27	51
F_1 Hybrid	1306	225	111	17	49

Table 4. Seed set and seed germination from the crosses made within the F_1 population, Spring, 1971.

Type of Cross	Flowers Examined	Good Seed	# Germ.	Seed Set (%)	% Germ.
Intrasp.	784	303	124	41	41
Sib	863	357	79	39	22

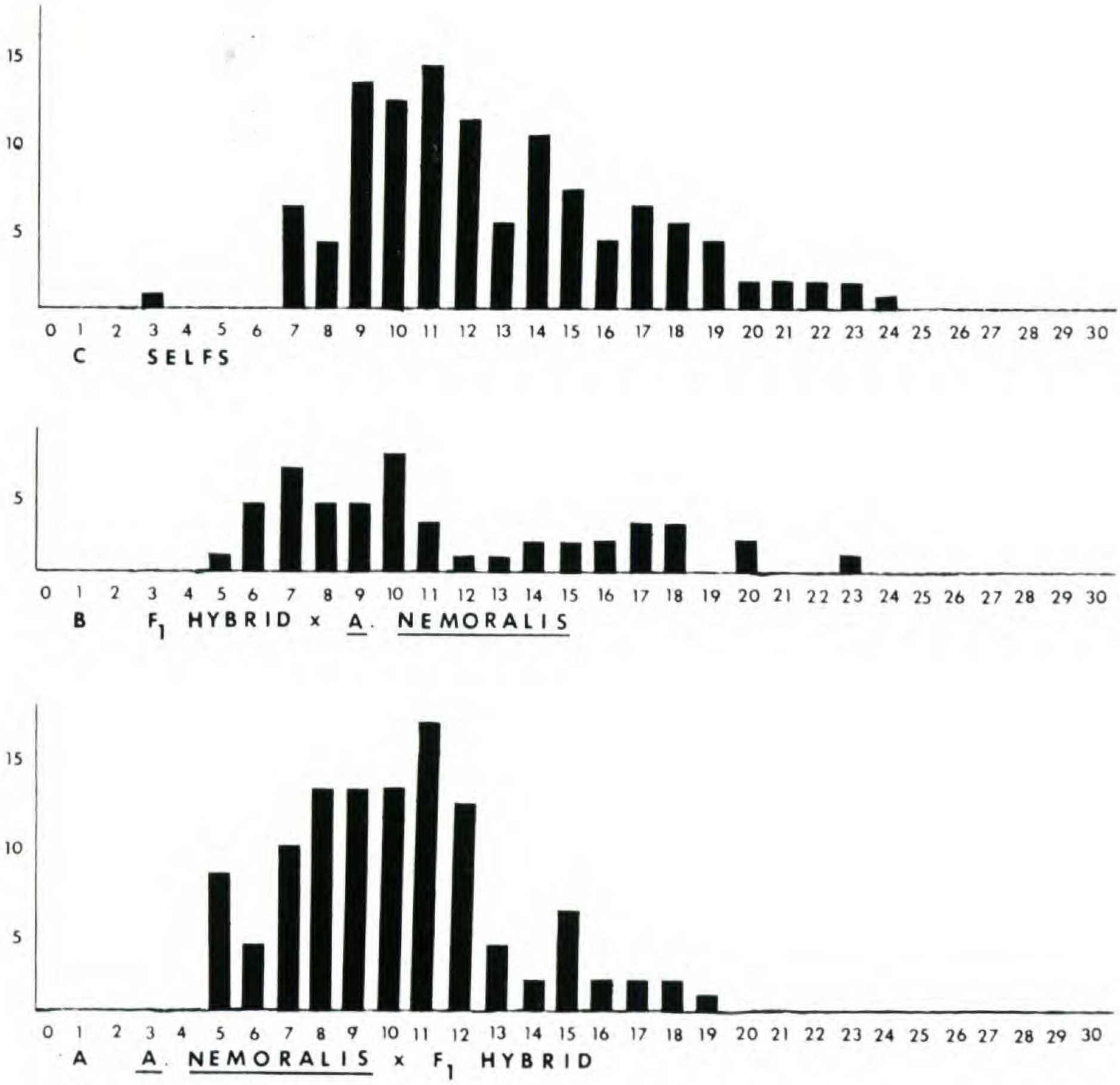


Figure 9. Morphological hybrid index of backcross and recombinant progeny of Asters collected Summer, 1972. The female parent is represented first under each histogram.

lower in progeny from sib-matings as compared with germination of progeny from intraspecific crosses (Table 4).

In the summer of 1972, 462 backcross and 110 recombinant progeny were collected and scored using the hybrid index of Pike (1970). These are represented in Figures 9 and 10. These data indicate that the range of variation assigned to *A. Blakei* by Pike contains backcross and recombinant progeny as well as hybrids. Chromatographs of sixty of these specimens showed chemical evidence for introgression. However, the backcross or recombinant progeny could not be identified on the basis of chemistry alone. Thus,

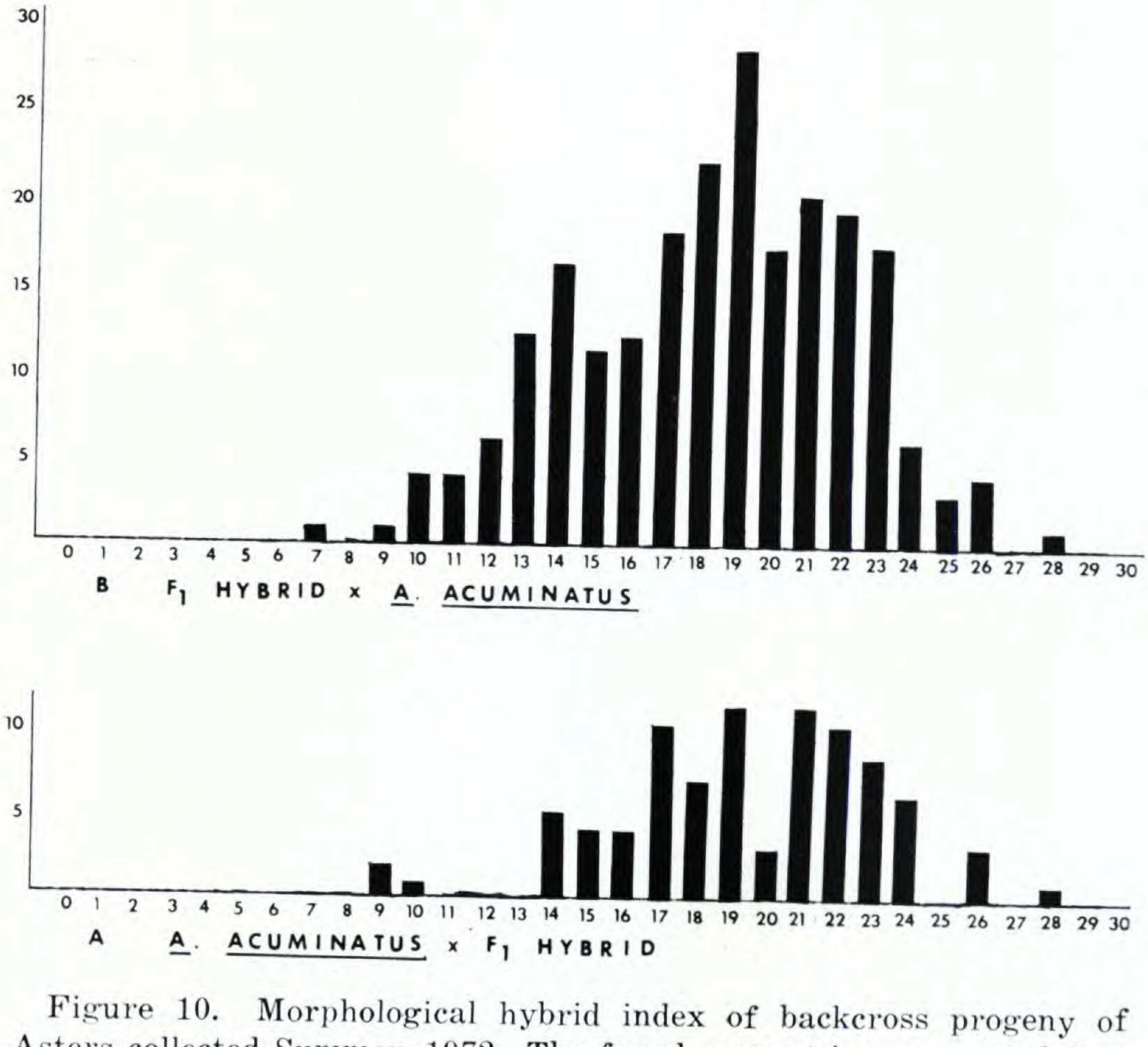


Figure 10. Morphological hybrid index of backcross progeny of Asters collected Summer, 1972. The female parent is represented first under each histogram.

chromatography might not be applicable in determining population structure within this aster complex, although this chemical technique has been successful in this regard in other genera, such as *Coreopsis* (Crawford, 1972) and *Baptisia* (McHale & Alston, 1964). Some of the backcross and recombinant progeny morphologically resembled some of the *A. Blakei* that the senior author has observed in his own collection as well as in Pike's collection and the specimens in the Herbarium at the University of New Hampshire.

The genetic results thus indicate that *A. acuminatus* and *A. nemoralis* can cross and produce a hybrid resembling *A. Blakei*. The hybrid can intercross with its parents and with

Table 5. Mean pollen stainability (%) of the backcross and recombinant progeny of asters collected in the summer of 1972.

Female Parent	plants counted	% stained pollen
A. nemoralis	52	81
F_1 Hybrid		
A. acuminatus	71	79
F_1 Hybrid		
A. nemoralis		
F_1 Hybrid	106	78
A. acuminatus		
F_1 Hybrid	36	80
F_2 Progeny	78	73

itself. The complex appears capable of forming hybrid swarms which indeed have been observed by Pike (1970) on some of the islands in the Bay of Fundy. He noted that some of these swarms show morphological skewness toward *A. nemoralis*. The data (Table 2) show that crossing between the two parental taxa favors *A. nemoralis* as the female parent. If this crossing occurred in nature, there should be more seeds of the F_1 produced on the *A. nemoralis* parent, and backcrosses would be more numerous in the wetter habitat of *A. nemoralis*. Progenies of the *A. nemoralis* backcrosses would tend to be favored in preference to *A. acuminatus* backcrosses in moist habitats. On the other hand, backcrosses between *A. acuminatus* and the F_1 hybrid were numerous and most were quite vigorous (Table 3). Crosses between *A. nemoralis* and the F_1 hybrid produced progeny which were numerous and vigorous when *A. nemoralis* was the female parent. A morphological skew was also noted toward *A. nemoralis* in the F_2 progeny (Figure 9c). It thus appears that under greenhouse conditions, introgression could go either way, while in nature, the in-

trogression is in the direction of *A. nemoralis*. The determining factor which would promote or inhibit introgression between *A. acuminatus* and *A. nemoralis* would thus be selection by the habitat.

CYTOLOGY

For root tip studies, 0.002 M 8-oxyquinoline was used as a pretreatment for 60-80 minutes at room temperature. The root tips were then stained and squashed in aceto-orcein according to the method of Huziwara (1957). Cover slips were smeared with Mayers albumin and dried over a flame to permit adhering of the preparation to the cover slip. Permanent mounts were made according to the technique of McClintock (1929) with the following modifications; the slide and cover slip were separated in 10% acetic acid using the method of Celeraier (1956); the cover slip was then passed through changes of 1:1, 1:3, and 1:9 acetic alcohol and two changes of 95% ethanol. The slide and cover slip were then recombined in diaphane. For meiotic studies, flower buds were fixed in 1:3 acetic alcohol. Flowers were dissected in a small vial containing 70% alcohol. Each flower suitable for analysis was placed on a slide in a drop of aceto-carmine stain. Permanent slides were made as described above. Chromosomes were observed under oil at 1125×. Photographs were taken with a Kodak camera mounted on a Spencer (A.O.) microscope with the preparation under oil at 1455×.

The chromosome number of these taxa was determined to be $2n = 18$. This was based on counts of mitotic and meiotic chromosomes of asters collected from Lake Ossipee, Gould Pond in Milton, New Hampshire, Great Wass Island, North Lubec, Maine, and Campobello Island in New Brunswick, Canada. A total of forty-three specimens gave good counts. The chromosome numbers of *A. Blakei* and *A. nemoralis* were new and were reported in the literature (Hill and Rogers, 1970). These specimens were not karyotyped. A representative plate of the mitotic chromosomes of *A.*

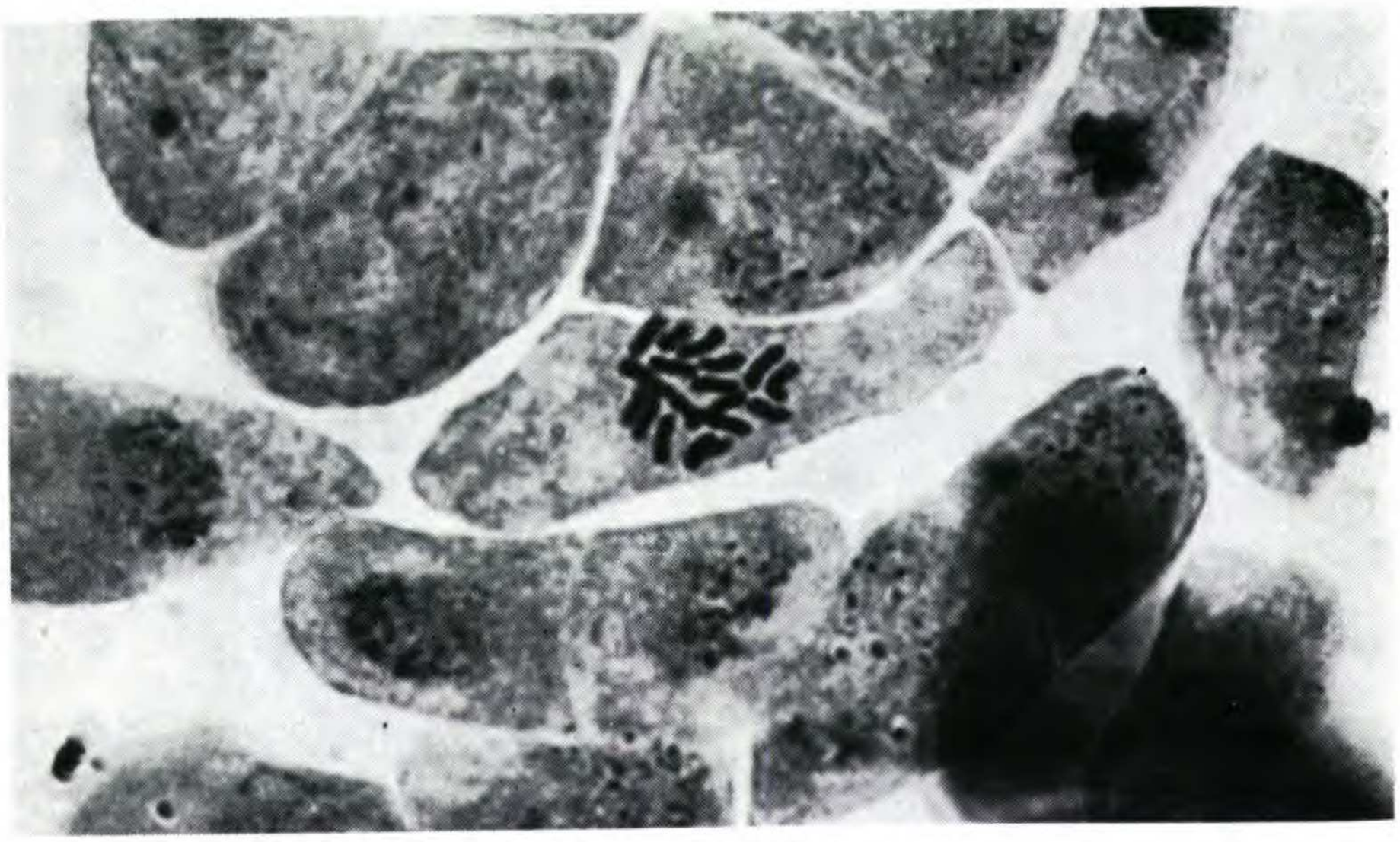
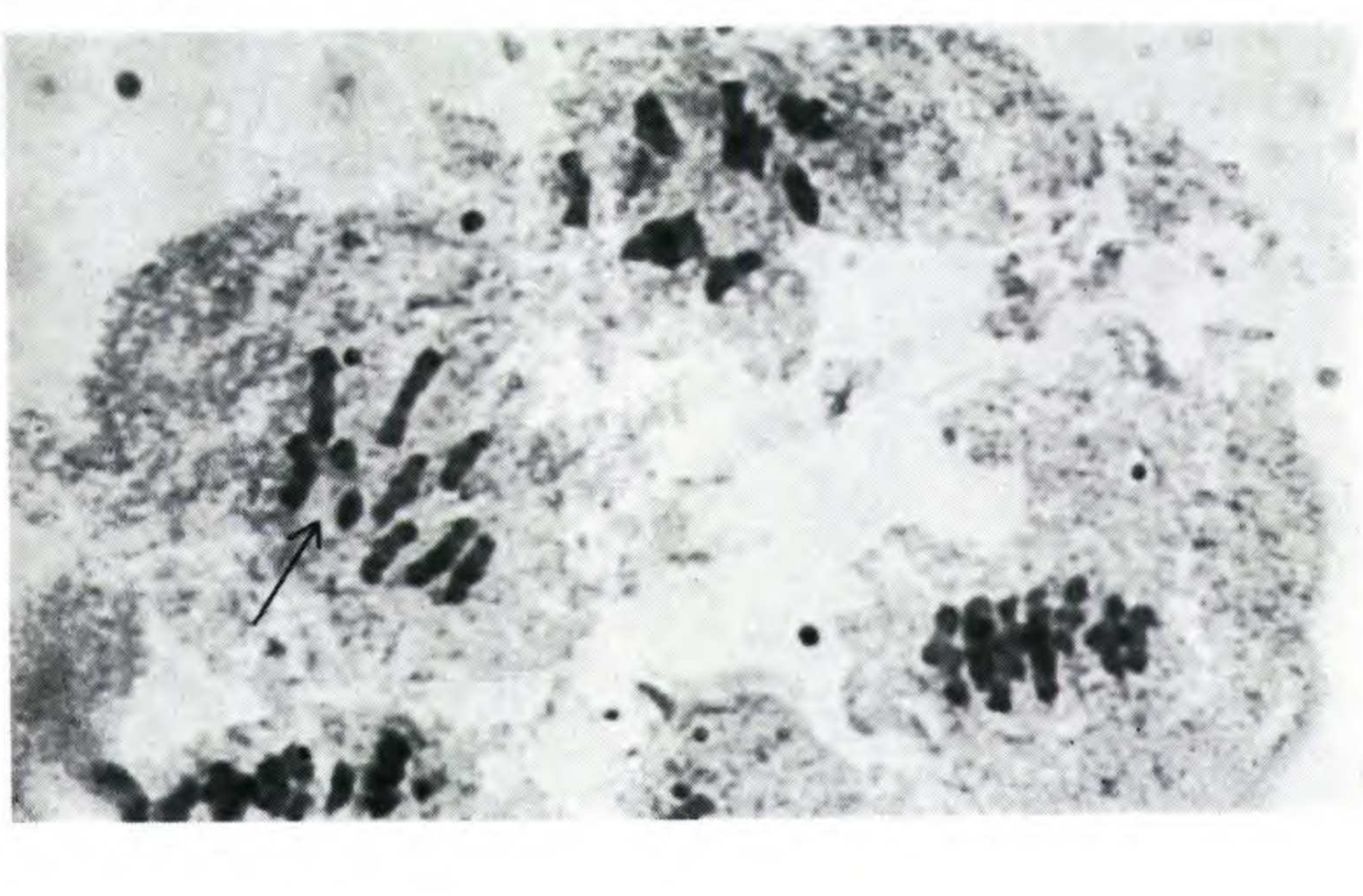


Figure 11. Mitotic chromosomes of *Aster nemoralis*, Metaphase. (above)

Figure 12. Meiotic chromosomes of *Aster Blakei*, Metaphase I. (below) The arrow indicates a loosely associated bivalent.



nemoralis is shown in Figure 11. A plate of the meiotic chromosomes of *A. Blakei* shows nine bivalents, one of them loosely associated (Figure 12). The behavior of the meiotic chromosomes of all three taxa was compared. Both parental taxa formed nine bivalents at meiosis. No irregularities were observed. Pairing in *A. Blakei* was regular although loose associations were occasionally noted. Some bridges and lagging were observed in Anaphase I. Meiotic stages on 239 plates were observed. Of these, 93% showed nine bivalents, and the remaining few demonstrated the irregularities referred to above. Similar observations were noted in the F_1 population.

Pollen from 41 F_1, 265 backcross, and 78 recombinant progeny were stained and scored. The pollen grains were stained with aniline blue in lactophenol, those staining dark blue being scored as fertile. The first 200 grains were scored for stainability under 10× magnification. The results for the backcross and recombinant progeny are listed in Table 5. The mean pollen stainability was lower in these progeny than was the mean stainability in the F_1 hybrid (89-90%) and parents (96-97%). The differences noted in Table 5 were not statistically different. Although it is obvious that a certain degree of hybrid breakdown has occurred, it appears that the F_1, backcross and recombinant progeny synthesized in the greenhouse are fertile.

CONCLUSIONS

The chemical evidence indicated that specimens identified as *A. Blakei* were clearly intermediates of *A. acuminatus* and *A. nemoralis*. Identification of *A. nemoralis*, *A. acuminatus* and *A. Blakei* could be done on the basis of phenolic examination alone. This is also true of the F_1 hybrids of the cross *A. nemoralis* × *A. acuminatus*. Backcross or recombinant progeny could be identified morphologically, but they could not be identified on the basis of chemistry alone.

The genetic evidence indicated that the hybrid of *A. acuminatus* and *A. nemoralis* was attainable and was in-

distinguishable from *A. Blakei.* The parental taxa can intercross with the F_1 hybrid and the F_1 hybrid can produce F_2 progeny. The production of a hybrid swarm in the greenhouse thus suggests the production of hybrid swarms in nature. The backcross and recombinant progeny were indistinguishable from some specimens of *A. Blakei.*

The cytological evidence demonstrated that the chromosome number for *A. Blakei*, *A. acuminatus*, and *A. nemoralis* was $2n = 18$. *A. Blakei* and the F_1 hybrid demonstrated regular meiosis most of the time. Pollen stainability with aniline blue in lactophenol was very high in both parental taxa, lower in the F_1 hybrid and even lower in the backcross and recombinant progeny. The evidence suggested that *A. Blakei* was a fertile species hybrid.

The data thus lead to a confirmation of Pike's (1970) hypothesis that A. *Blakei* is a hybrid of *A. acuminatus* and *A. nemoralis.* It is always difficult to assess the exact size of a species population in terms of the number, location, and size of individual sub- or local populations over a species range. Our sample sizes reflect only a small area that covers the range of *A. Blakei.* Keeping this in mind, we wish to suggest that *A. acuminatus* and *A. nemoralis* are semi- or incipient species. They remain isolated by geography but cross within their overlapping ranges when the opportunity is presented. The major isolating barrier between the species would be the absence of hybrid or recombinational habitats when interspecific crossing occurs.

ACKNOWLEDGMENTS

Thanks are extended to Dr. Radcliffe B. Pike for his efforts in collecting the parental asters used in the genetic portion of this study, for his help in cultivating asters from seed and for his aid in locating the asters at Ponds Point, Great Wass Island. The hospitality of the summer residents near Ponds Point is also greatly appreciated. Thanks are also due to Dr. Albion R. Hodgdon for his help in locating the populations at Lake Ossipee and Lake Winnisquam. We

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